

AMENDMENT TO SPECIFICATION

Please amend the paragraph beginning on page 36, line 29 as follows:

Abs. Human polyclonal IgG was prepared by affinity chromatography on Protein G-Sepharose (Amersham Pharmacia) from sera of 6 healthy human subjects (lab codes 1086, 1087, 1088, 1091, 1092, 1518). IgG from pooled serum from 8 BALB/c mice (4-5 wk) was obtained similarly. Preparation of polyclonal Abs by hyperimmunization with synthetic Cys-gp120(421-436) (KQIINMWQEVGKAMYA; SEQ ID No. 5; residues 421-436 of gp120 HIV SF2 strain) conjugated to KLH is described in (13). Polyclonal Abs to exEGFR were raised by immunizing female BALB/c mice (5-6 wk) intraperitoneally with exEGFR (10 .mu.tg/injection) on days 0, 27 and 41 in RIBI adjuvant and with A431 tumor cells (10.sup.7 cells in saline) on day 14. Monoclonal Abs to exEGFR (clones C225, H11, and C111.6) were purchased from Labvision (Fremont, Calif.). A control monoclonal anti-BSA IgG (clone BGN/H8) was from Biogenesis (Kingston, N.H.) Single chain Fv constructs (N=15) were picked randomly from a human Fv library derived from lupus patients described in (11) (MM series clones; 12, 14, 18, 20, 24, F1, F2, F4, F5, F6, F7, F11, F12, F14, F17, F18). The scFv proteins were purified to electrophoretic homogeneity (27 kDa band) by metal affinity chromatography on Ni-NTA columns (11). Expression levels were 0.3-5.7 mg/liter bacterial culture. The library contains diverse scFv clones determined by nucleotide sequencing (11), assuring a broad sampling of Ab V domains. One of the scFv clones examined in the present study, MM-F4, was sequenced (GenBank #AF522073) and its VL and VH domains were determined to belong to families 1 and 1, respectively, and the germline gene counterparts were V1-13 and VH1-2, respectively. Confirmation of scFv band identities in SDS-electrophoresis gels was by immnunoblotting using a monoclonal Ab to c-myc (10).

Please amend the paragraph beginning on page 52, line 24 as follows:

MAb YZ20 did not cleave biotinylated BSA or the extracellular domain of the epidermal growth factor (exEGFR), indicating selectivity for gp120 (FIG. 18A). Attempts to identify the bonds cleaved by MAb YZ20 were unsuccessful. N-terminal sequencing of the 55 kD and 50 kD bands yielded identical sequences (TEKLWVTVYY; SEQ ID No. 7), corresponding to the N terminal residues of gp120. Sequencing of the 15 kD band from the YZ20 reaction mixture did not yield detectable phenylthiohydantoin derivatives of amino acids, possibly due to a blocked N terminus. Identification of the 27 kD gp120 fragment is complicated because of its configuration with the Ab light chain in reducing gels. As identification of the

precise bonds in gp120 cleaved by the MAb was not central to the present study, we turned to the use of model peptide substrates for determination of scission bond preferences. A fluorimetric assay was employed to determine MAb-catalyzed cleavage of the amide bond linking aminomethylcoumarin to the C terminal amino acid in a panel of peptide-MCA substrates (FIG. 18B). The peptide-MCA substrates are used at excess concentration (200 .mu.M), permitting detection of even weakly cross-reactive catalytic Abs. Selective cleavage at Arg-MCA and Lys-MCA was observed, with no evident cleavage on the C terminal side of neutral or acidic residues. To confirm that the rate differences are due to recognition of the basic residue at the cleavage site (as opposed to remote residues), we studied two tripeptide substrates identical in sequence except for the N terminal residue at the scission bond, Gly-Gly-Arg-MCA attd Gly-Gly-Leu-MCA. The former substrate was cleaved at detectable levels by Ab YZ20 [0.31.+-.0.01 (s.d.) .mu.M AMC/19 h/.mu.M IgG], whereas the fluorescence intensity in reaction mixtures of the latter substrate and the Ab was statistically indistinguishable from background values observed in assay diluent [0.02.+-.0.04 .mu.M AMC/19 h/.mu.M IgG; P>0.05; student's t-test; unpaired; FIG. 18C]. The basic residue preference is consistent with the presence of positively charged amidino groups neighboring the phosphonate groups in the immunogen (III) and selective cleavage on the C terminal side of Arg/Lys residues by germline encoded proteolytic Abs observed previously (21,22).